

EXHIBIT A

2/15/00

Clean DNA first

Run agel

there are just a

little DNA in expect size

So improve Digest

Digest

DNA 40ul

H₂O 42.5ul

Buffer 10ul

EcoRI 2.5ul

10ul

2.5ul

Govermoy 270

GENOSYS

HAPDAYS

HAPDAYS

HAPDAYS

HAPDAYS

GENOSYS

HAPDAYS

HAPDAYS

HAPDAYS

HAPDAYS

GENOSYS

HAPDAYS

HAPDAYS

HAPDAYS

HAPDAYS

→ primer

New

Digest - to check differential digest procedure for clean gene & detection

1039 or 1153 DNA

1ul

10x buffer

H₂O

1ul

2.5ul

0.5ul

enzyme

30 digen

Sau 3A 2

Dpn I

Hpa I

bott

unmethylated gene

unmethylated gene

Digest 37°C 4 hr

Read and Understood By

Anne J. J.

Sinner

Signed

Inverse PCR procedure

Use 100 µl	ELORI digest	Set up	3 Digestions
1039 ELORI int DNA	1	2	5
10x buffer	5	5	5
Ta Ligase (NEB)	0.5	0.5	0.5
H ₂ O	33.5	33.5	33.5

16°C overnight
for PCR

heat inactivate
swirl

2/1/100

Run a gel to detect differential digest procedure
for dam gene.

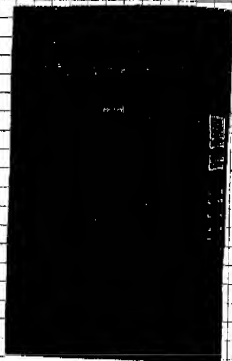
Ladder ① 1039 Sam3AI ② 1039 MboI ③ 1039 DpnI
② D153 ④ D153 ⑤ D153

There ~~one~~ some is
dam gene in D153.

Sam3AI → digest GATC
both methylated and unmethylated

MboI → digest GATC
only unmethylated

DpnI → digest GATC
only methylated.



⑥ ⑤ ④ ③ ② ①

Read and Understood By

Omara J. [Signature]